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COLONISATION OF PINE WOOD (*PINUS SILVESTRIS* L.) IN WATERLOGGED GLEYSOIL BY MICROORGANISMS

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During four and eight weeks of contact of pine wood (*Pinus silvestris* L.) with gleysoil under anaerobic conditions colonization of wood by bacteria, actinomycetes and *Deuteromycotina* was observed. It was found after eight weeks that aerobic cultures of cellulolytic bacteria: *Bacillus* sp., mesophilic and thermophilic *Clostridium* sp., cellulolytic actinomycetes *Micromonospora* sp. cellulolytic fungi *Aspergillus* sp. and *Penicillium* sp. could colonize pine wood. The following bacteria also were present inside wood: nitrifying, ammonifying, sulphate reducing *Clostridium* sp., iron reducing anaerobic bacteria and iron oxidising *Thiobacillus ferrooxidans* (only after four weeks).

INTRODUCTION

There is a large class of uses of wood in direct contact with ground. Durability of wood in such a use is rather low. Efficiency of performance of preservatives used for wood protection depends on the type of soil with which wood is used in contact. Copper-based compounds are among to the wood preservatives used most commonly. Results of Morris [18] confirmed the earlier hypotheses [25] that ferrous ions migrating from the soil to the wood reduce the efficiency of performance of copper-based preservatives. Reduction of ferric compounds to ferrous ones (Fe^{2+} to Fe^{3+}) occurs in gley process, in which anaerobic bacteria play a part. Dickinson [8] presented comparison of performance of CCA-protected wood in field tests in many countries. This comparison of results of 10-years investigations of efficacity of wood preservatives in different areas of the Globe showed that the stakes placed at Westham Island in British Columbia (Canada) were subject to the fastest decay. This field site is periodically flooded with water (in winter – spring). Ruddick and Kundzewicz (24) confirmed the dominant role of bacteria in iron uptake

by the wood in the Westham Island soil. Presence of Fe reducing bacteria has been found in gley soil from the area of Konstancin-Jeziorna [15].

Bacteria along with fungi bring about significant losses to wood every year. Recent studies employing scanning and transmission electron microscopy in studies of bacterial degradation of pine wood (*Pinus radiata*) [27]. Bacterial degradation patterns have been described by various names, e.g.: tunneling [7, 20], cavitaton [21], burrowing [19], cavity formation, erosion and erosion zones [6, 12, 28].

Decomposition of lignin by bacteria of the genus *Clostridium* was described by Emtsev [11]. The transformation of lignin by pure cultures of bacteria: *C. pasteurianum, C. butyricum, C. acetobutylicum* and *C. butylicum* was studied. Significant roles of presence of anaerobic methanogenic and sulphate reducing bacteria in mining timber and drainage water from a gold mine were described Abraham et al. [1]. The methanogens were isolated from the mine environment together with sulphate-reducing and nitrate-reducing bacteria.

In the so far considerations on the role of bacteria in penetration of iron into wood the microbiological aspect has been neglected. The proofs of participation of bacteria in stimulating the process of iron migration to the wood are definitive. However the bacteria have not been identified, thus one cannot distinguish their particular species. Attempts to obtain degradation of lignified wood cell walls in laboratory by pure culture of bacteria have not been successful so far [27].

The present study is an attempt to determine which microorganisms can be responsible for significantly lower durability of wood used in contact with gleysoil.

MATERIALS AND METHODS

Pine (*Pinus silvestris* L.) wood samples $50 \times 25 \times 15$ mm were inserted vertically into gleysoil in the 100 l container (bioreactor) with a light covering of surface water. Top surfaces of samples were in the soil, at the depth of eight cm. They were kept in the soil in room temperature for periods of four (I) and eight (II) weeks respectively in two sets of experiments. Soil samples were taken from Konstancin-Jeziorna area, from the depth of 50-60 cm. Detail physical and chemical characteristics of the soil were described by Kundzewicz and Witter [16]. At the end of each period wood samples were removed and the presence of anaerobic bacteria and aerobic bacteria, actinomycetes and fungi were investigated separately in the soil and in the wood. Samples of soil were taken (a) from the surface of wood and (b) in the distance of 10 cm from the location of samples. Chips of wood were taken from the surface of the wood samples (c) before sterilization with ethanol and short contact with flame and (d) after sterilization, and from different sections of wood samples: (e) 1 mm, (f) 2 mm and (g) 3 mm.

The total number of soil bacteria, actinomycetes and fungi were investigated using the following agar media: by Bunt and Rovira [5] with 50 μg cm^{-3} of actidione, by Waksman [29] for actinomycetes with starch, by Martin [17], with 50 μg cm^{-3} of streptomycine, respectively.

For microbiological analysis the following liquid media were used:
- Dubos medium [10] with stripes of filter paper for areobic mesophilic cellulolytic bacteria;
- CM3 medium by Weimer and Zeikus [30] with stripes of filter paper for mesophilic and termophilic anaerobic cellulolytic bacteria;
- Coppier and de Barjac medium for nitrifying bacteria [13];
- de Barjac medium for denitrifying bacteria [13];
- medium for amonifying bacteria (with Vinogradsky salts) [3];
- *Clostridium* sulphate reducing medium [22];
- Nfb medium by Döbereiner et al. [9] for N_2 fixing *Azospirillum* sp. bacteria;
- Postgate modification of Baars's medium (23) for iron sulphate reducing bacteria (anaerobic mesophiles);
- Casida et al. [26] for iron reducing bacteria (aerobic mesophiles).

Soil samples: 1 g for (a) or 10 g for (b) – wet weight were suspended into 9 or 90 cm^3 of sterile water and mixed with shaker for 15 min. After serial 10-fold dilutions in sterile water suspensions were spreaded onto the liquid media defined above. The incubation temperature was 28 °C for isolation of mesophiles, 60 °C for thermophiles and 20 °C for fungi. The incubation period was three days for mesophilic bacteria growing on Bunt and Rovira [5] plates and seven days for the rest of microorganisms. The average number of microorganisms isolated on three of five plates was considered the viable cell number. Results of presence of cultures in liquid media were designated on basis of a visible density and morphological observations as „+" or" – " (with or without growth). In the analysis of the first phase of nitrification (nitritation), Griess-Ilosvay [14] reagent was used for identification of nitrite presence. Diphenylamine and 96% sulphuric acid solution were used to analyze denitrification according to de Barjac [13]. Additionally, the presence of gas sampling tubes was noted and pH of denitrifying culture were measured. Identification of bacterial ammonification process was obtained after positive reaction with Nessler's reagent (2. Pine wood chips (0.1 mg) were added into all mentioned liquid media and three pieces of wood were placed on agar media and incubated using the same methods as with soil.

Dry weight of soil was measured after oven-drying in 103°C to constant mass. Moisture of soil were measured basing on following formula:

$$\text{Soil moisture} = \frac{W_t - W_o}{W_o}$$

when W_t – wet mass, W_o – dry mass.

Light microscopical observations were provided for morphology and motility of bacteria. Bacteria were stained by Gram's method. For the classification of bacteria and actinomycetes Bergey's systematic was used [4]. For preliminary diagnosis of fungi colonies were inoculated on Czapek-Dox agar blocks (29) and Martin's medium [17]. Fungi were microscopicaly observed after 3-7 days of incubation and were determined according to Barnett's classification [3]. Cellulolysis of bacteria, actinomycetes and fungi were investigated on/in media containing filter paper or carboxymethylcellulose (CMC) as a source of cellulose.

RESULTS AND DISCUSSION

A significant difference was observed between moisture of the soil in bioreactor collected at the surface of wood (121%) and in the distance 10 cm from wood (282.76%). The soil pH measured in water was (a) 6.41 in the case of soil adjacent to the wood surface and (b) 6.65 for soil from 10 cm from wood samples and 5.85 in KCl solution for both soils. Colonization of pine wood (*Pinus silvestris* L.) stored in soil in nearly the anaerobic conditions by mesophilic and termophilic soil bacteria, actinomycetes and fungi was observed. The presence of these microorganisms was noted not only on the surface

Table 1

Colonization of pine wood after gleysoil exposure by bacteria, actinomycetes and fungi and their presence in gleysoil

Zasiedlenie drewna sosny przechowywanego w glebie glejowej przez bakterie, promieniowce i grzyby oraz ich obecność w glebie

Microorganisms Mikroorganizmy	Growth of microorganisms Wzrost mikroorganizmów													
	soil gleba		chips of pine wood ścinki drewna											
	a		b		c		d		e		f		g	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Bacteria Bakterie	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Actinomycetes Promieniowce	+	+	+	+	+	+	+	+	+	+	+	–	–	–
Fungi Grzyby	+	+	+	+	+	+	+	+	+	+	–	–	–	–

Note: + growth; – no growth

I – four weeks exposure of wood in gleysoil

II – eight weeks exposure of wood in gleysoil

Samples of soil from: a – the surface of wood, b – in the distance of 10 cm from the location of samples. Chips of wood from: c – the surface of wood before sterilization, d – after sterilization, e – 1 mm section, f – 2 mm section, g – 3 mm section.

Notka: + wzrost; – brak wzrostu

I – cztery tygodnie przechowywanie drewna w glebie

II – osiem tygodni przechowywania drewna w glebie

Próbki gleby pobrane z: a – powierzchni drewna, b – z odległości 10 cm od próbki drewna. Skrawki drewna: c – z powierzchni drewna przed strylizacją, d – po sterylizacji, e – z głębokości 1 mm, f – z głębokości 2 mm, g – z głębokości 3 mm.

Table 2

Colonization of pine wood after gleysoil exposure by aerobic and anaerobic bacteria and their presence in gleysoil

Zasiedlenie drewna sosny przechowywanego w glebie glejowej przez tlenowe i beztlenowe bakterie oraz ich obecność w glebie

Type of bacteria	Growth of bacteria													
	soil				chips of pine wood									
	a		b		c		d		e		f		g	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Cellulolytic:														
– mesophiles:														
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Clostridium sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
– termophiles:														
Clostridium sp.	+	+	+	+	+	+	+	+	+	+	–	–	–	–
Nitrogen fixing:														
Azotobacter sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Azospirillum sp.	+	+	+	+	+	–	–	–	–	–	–	–	–	–
C. pasterianum	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrifying aerobes	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Denitrifying aerobes	+	+	+	+	+	+	–	–	–	–	–	–	–	–
Ammonifying aerobes	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulphate reducing														
Clostridium sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Iron sulphate														
reducing aerobes	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Iron reducing														
Clostridium sp.	+	+	+	+	+	+	+	+	+	+	+	+	–	–
Iron oxidizing														
Thiobacillus														
ferrooxidans	+	+	+	+	+	–	+	–	+	–	+	–	+	–

Designation as on Table 1 – Oznaczenia jak w tabeli 1

of wood, but also inside (Table 1). The aerobic, mesophilic bacteria, actinomycetes and fungi were found in both soil samples (a and b) and in wood to 1 mm depth. The aerobic actinomycetes were still observed at the layer of 2 mm depth, however only after four weeks exposure (I). The only group of organisms determined at the depth of 3 mm was bacteria. In the class of aerobic mesophiles, according to Table 1, the activity of penetration from soil to wood can be ranked in the following order: bacteria, actinomycetes and fungi. The dominant genus of actinomycetes was cellulolytic strain *Micromonospora* sp. The isolated fungi were determined as a *Deuteromycotina.*

In Table 2 the results of possibility of penetration from soil to pine wood are presented for the different physiological groups of bacteria: mesophiles and termophiles, aerobes and anaerobes. The following sporeforming mesophilic, cellulolytic bacteria were observed aerobic mesophilic bacteria (temperature of incubation was 28°C), mainly, *Bacillus* sp., aerobic and anaerobic mesophiles (37°C) and thermophiles (60°C), mainly *Clostridium* sp. Cultures of *Bacillus* sp.

produced transparent slime was present, similarly like cultures of *Clostridium* sp. in every investigated layer of wood. However, the thermophilic *Clostridium* sp. cultures were present only in the 1 mm layer. Presumably, the slime could have significant role in adhesion on wood surface and the movement of microorganisms into wood.

Among the N_2 fixing bacteria, determined only *Clostridium pasterurianum* cultures were present in 3 mm layer of wood (Table 2). Bacteria *Azospirillum* sp. which were present in soil and on the wood surface before steriliation after four weeks of exposure, were absent on the wood surface after eight weeks of exposure and inside wood. It is worth noting that both isolated cultures of bacteria (*Clostridium pasteurianum* and *Azosporillum* sp.) are not cellulolytic. Absence of *Azotobacter* sp. bacteria in soil and wood confirm gleysoil is not a suitable environment for such a low soil pH sensitive strict aerobic strain.

The nitrifying, ammonifying, *Thiobacillus ferrooxidans* and sulphate reducing *Clostridium* sp. could penetrate from soil into the deepest (3 mm) layer of pine wood (Table 2). Denitrifying bacteria were observed at both soil samples (a, b) and on non-sterilized surface of wood (I and II) and were not noted on sterile wood surface and inside wood samples. Test for indentification of facultative aerobic iron sulphate reducing bacteria shown their presence in both soil samples (a, b) and absence in wood (c to g). However iron reducing (Fe_2O_3) anaerobic bacteria were present in the soil (a,b) and in wood, except 3 mm layer (I and II). Iron oxidating ($FeSO_4$) bacteria *Thiobacillus ferrooxidans* appeared in soil samples (a, b) and in every wood layers after four weeks of exposure (I), but never after eight weeks (II). Perhaps more intensive colonization of anaerobic microorganisms after eight weeks provided poor condition for iron oxidation.

Table 3

The total number of mesophilic bacteria, actinomycetes and fungi in gleysoil samples
Ogólna liczba mezofilnych bakterii, promieniowców i grzybów w próbkach gleby glejowej

Soil samples Próbki gleby	The total number of microorganisms Ogólna liczba mikroorganizmów g^{-1}d.w.$^{-1}$ g^{-1}s.m.$^{-1}$		
	bacteria bakterie	actinomycetes promieniowce	fungi grzyby
I a	3.0×10^5	3.0×10^4	8.0×10^3
I b	4.4×10^5	1.2×10^5	2.1×10^4
II a	3.6×10^5	1.6×10^5	3.0×10^3
II b	5.4×10^5	1.6×10^5	1.0×10^4

Designation as on Table 1 – Oznaczenia jak w tabeli 1

Results of total number of viable microorganisms in soil were presented in Table 3. No significant differences of the total number of aerobic mesophilic bacteria were observed independently of time (I and II) and location of soil

samples. A slight tendency of increase of the total number of actinomycetes was observed between soil from surface of wood ofter four weeks (I): 3.0×104 and after eight weeks (II): 1.6×105 5 times more. *Micromonospora* sp. was determined as a dominating cellulolitic actinomycetes isolated from the soil. The total number of fungi in each variant of soil samples were almost the same. A very small decrease of the total number of fungi growing in soil adjacent to the surface of pine wood was noted after eight weeks of it's storing-from 8.0×103 to 3.0×103; that is about 2.6 times less.

Table 4

Colonization of pine wood after gleysoil by *Deuteromycotina* fungi and their presence in gleysoil

Zasiedlenie drewna sosny przechowywanego w glebie glejowej przez grzyby z klasy *Deuteromycotina* oraz ich obecność w glebie

Soil samples	*Deuteromycotina*	
	I	II
a	*Aspergillus* sp. *Bortytis* sp. *Penicillium* sp.	*Aspergillus* sp. *Penicillium* sp.
b	*Amblyosporium* sp. *Aspergillus* sp. *Cladosporium* sp. *Gonadobotrys* sp. *Penicillium* sp.	*Alternaria* sp. *Aspergillus* sp. *Cladosporium* sp. *Fumago* sp. *Gliocladium* sp.
c	*Penicillium* sp.	*Penicillium* sp.
d	*Aspergillus* sp.	*Penicilium* sp.
e	*Aspergillus* sp.	*Penicillium* sp.
f	–	–
g	–	–

Designation as on Table 1 – Oznaczenie jak w tabeli 1

The results were presented in Table 4 have shown, that pine wood was infected by only two cellulolytic fungi: *Aspergillus* sp. (after four weeks) and *Penicillium* sp. (after eight weeks). The following *Deutromycotina* were isolated in the soil adjacent surface of wood samples: *Aspergillus* sp., *Botrytis* sp., *Penicillium* sp. In the soil in 10 cm of distance from wood were grewn following *Deutromycotina: Amblyosporium* sp., *Aspergillus* sp., *Cladosporium* sp., *Fumago* sp., *Gliocladium* sp. and *Gonadobotrys* sp.

Presented study can be regarded as a preliminary step in research on wood colonization by microoorganisms (particularly anaerobic mesophilic and termophilic bacteria). Determination of the role of microorganisms participating in wood colonization and interaction between them during contact with gleysoil requires detailed investigations. Study of mechanism of acceleration of wood decay in gleysoil seems very interesting. Examination of treated wood in order to create preservative system suitable for wood used in contact with gleysoil should be a necessary study component.

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MIKROORGANIZMY ZASIEDLAJĄCE DREWNO SOSNY (*PINUS SILVESTRIS* L.) W KONTAKCIE Z GLEBĄ OPADOGLEJOWĄ

Streszczenie

Próbki drewna po 4 i 8 tygodniowym przebywaniu w glebie glejowej w warunkach zbliżonych do beztlenowych były zasiedlane przez bakterie, promieniowce i grzyby z podgromady *Deuteromycotina*. Po ośmiu tygodniach ekspozycji stwierdzono w próbkach drewna obecność tlenowych, celulolitycznych bakterii, głównie *Bacillus* sp., mezofilnych i termofilnych bakterii beztlenowych, głównie *Clostridium* sp., celulolitycznych promieniowców i celulolitycznych grzybów *Aspergillus* sp. i *Penicillium* sp. Ponadto wykryto w drewnie obecność bakterii: nitryfikacyjnych, amonifikacyjnych, *Clostridium* sp. redukujących siarczyny, beztlenowych bakterii redukujących związki żelaza oraz *Thiobacillus ferrooxidans* (tylko po 4 tygodniach).

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